2. Document ID: US 20010034435 A1

L22: Entry 2 of 100

File: PGPB

Oct 25, 2001

PGPUB-DOCUMENT-NUMBER: 20010034435
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010034435 A1

TITLE: Process and equipment for plasmid purification

PUBLICATION-DATE: October 25, 2001
US-CL-CURRENT: 536/23.1; 435/270

APPL-NO: 09/ 774284
DATE FILED: January 29, 2001

RELATED-US-APPL-DATA:
RLAN
RLFD
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09774284
Jan 29, 2001
ABANDONED
A1
US
08887673
Jul 3, 1997
US
60022157
Jul 19, 1996

IN: Nochumson, Samuel, Durland, Ross, Yu-Speight, Audrey, Welp, John, Wu, Kuoewi, Hayes, Rexford

AB: A scalable alkaline lysis process, including procedures and devices for the isolation of large quantities (grams and kilograms) of plasmid DNA from recombinant E. coli cells. Effective, controllable, and economical operation, and consistent low level of host chromosomal DNA in the final plasmid product. Involves a series of new unit operations and devices for cell resuspension, cell lysis, and neutralization.

L22: Entry 2 of 100

File: PGPB

Oct 25, 2001

DOCUMENT-IDENTIFIER: US 20010034435 A1
TITLE: Process and equipment for plasmid purification

BSTX:
[0008] Once the plasmid DNA is extracted from the lysed cells its purification has become a routine and important procedure for the molecular biologist. However, the scale for these purifications, often referred to as "mini-preps", is usually less than about 1 milligram of plasmid DNA. These small scale preps isolate plasmid DNA from the supernatant of lysed bacterial cells using a variety of techniques, such as ethanol precipitation. For slightly larger scale preparations, the primary techniques employed use cesium chloride centrifugation, binding and eluting to silica resins (in the presence of chaotropic salts) or binding and eluting with various anionic chromatography resins. In addition, other techniques are sometimes used in combination with the resins mentioned, e.g., PEG and/or alcohol precipitation, RNase treatment, and phenol/chloroform extraction. There are also some plasmid purifications performed using analytical HPLC, in particular reverse phase HPLC to separate different plasmid forms using organic solvent systems.

59. Document ID: US 5561064 A

L22: Entry 59 of 100

File: USPT

Oct 1, 1996

US-PAT-NO: 5561064
DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA
DATE-ISSUED: October 1, 1996

US-CL-CURRENT: 435/320.1; 435/259, 435/91.1

APPL-NO: 8/ 192151
DATE FILED: February 1, 1994

IN: Marquet; Magda, Horn; Nancy, Meek; Jennifer, Budahazi; Gregg

AB: The invention relates to a method for producing plasmid DNA, comprising the steps of: (a) lysing cells containing the plasmid DNA to obtain a lysate; (b) treating the lysate by a means for removing insoluble material to obtain a solute; and (c) applying the solute to differential PEG precipitations and chromatography to purify the plasmid DNA. In other embodiments of the invention, the plasmid DNA is produced with GRAS reagents; the plasmid DNA is produced in the absence of enzymes; the plasmid DNA is produced in the absence of organic extractants; the plasmid DNA is produced in the absence of mutagens; the lysing, treating and applying steps are scalable to result in the large scale manufacture of the plasmid DNA; and the lysing, treating and applying steps result in the generation of pharmaceutical grade material.

L22: Entry 59 of 100

File: USPT

Oct 1, 1996

DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA

DEPR:
This manufacturing process results in the production of milligram, gram and kilogram quantities of pharmaceutical grade plasmid DNA. In general, the method involves: lysing cells (e.g., bacteria, yeast, fungi, mammalian, insect or other cells) obtained through shake flask culture, bioreactor or fermentor propagation containing the plasmid DNA to obtain a crude lysate; concentrating and separating a partially purified DNA intermediate significantly enriched in plasmid DNA from host contaminants such as cell debris using filtration, centrifugation, any form of chromatography and/or differential precipitation methods; removing remaining contaminants such as proteins, RNA, lipids, and chromosomal DNA from the partially purified DNA intermediate by chromatography and/or differential precipitation methods; accomplishing a fine separation of residual contaminants and remaining forms of DNA by chromatography and/or differential

precipitations; removing air-borne
microbes introduced during processing by sterilizing the desired plasmid fraction, and aseptically
filling vials for appropriate delivery of a pharmaceutical dosage form.

DEPR:
Plasmid VCL-1005 was transformed into a standard strain of DH10B E. coli (BRL, Gaithersburg, Md.).
Cells were grown in a 10 L fermentor (Braun) using standard TB medium. At the end of the exponential
phase, cells were harvested by centrifugation and lysed by alkaline lysis (without the use of
lysozyme). Cell debris were separated by filtration. Plasmid DNA was precipitated and fractionated
by standard low pressure chromatography. Appropriate fractions were pooled and the DNA was
formulated. The concentration was adjusted and the DNA was sterile filtered and filled into sterile
vials. Using the process of the invention, sufficient material was produced and purified for
pre-clinical and clinical studies that met the criteria of identify, purity, potency and safety of
pharmaceutical products derived from E. coli as defined by the FDA.

DETL:

Disclosed Pharmaceutical
Procedure Laboratory Method Manufacturing Process

Cell Lysis Employs
Lysozyme, No Enzymes, Alternate Tris, SDS Buffer such as Sodium Acetate, Alkaline pH and/or Tween
.RTM. 80 instead of SDS; only GRAS reagents used Removal of Cell Centrifugation Filtration or Debris
Centrifugation Removal of Host Uses Animal Derived High Salt Precipitation, Cell Derived Enzymes,
Uses Organic PEG Precipitation of Contaminants: Solvents Non-Plasmid Contaminants RNA, Protein,
Lipid, DNA Plasmid Enriched Ethanol or Similar PEG Precipitation of Crude Lysate Alcohol Plasmid DNA
Purification of High-speed Chromatography; Column Plasmid DNA Centrifugation; is Scalable; No Toxic
CsCl/EtBr Gradients Reagents

72. Document ID: US 5217593 A

L22: Entry 72 of 100 File: USPT Jun 8, 1993

US-PAT-NO: 5217593
DOCUMENT-IDENTIFIER: US 5217593 A
TITLE: Nucleic acid purification system and method
DATE-ISSUED: June 8, 1993

US-CL-CURRENT: 204/457; 204/608

APPL-NO: 7/ 911515
DATE FILED: July 10, 1992

PARENT-CASE:
REFERENCE TO RELATED APPLICATION The present application is a continuation-in-part of co-pending
application Ser. No. 07/668,856, now U.S. Pat. No. 5,139,637 filed Mar. 13, 1991 and entitled
PLASMID PURIFICATION SYSTEM AND METHOD.

IN: MacConnell; William P.

AB: A process for the purification of DNA and the like comprises a housing having walls
forming a reservoir having a chamber for containing a buffer solution, means for circulating a
buffer through the reservoir, a disposable cassette within said chamber having first means
including a gel for defining a first path extending between an inlet end and an outlet end, a
well for introducing a bacterial sample into the path at said inlet end thereof, and a second
path intersecting the first path via an elution chamber, having a collection chamber including
an elution window at said outlet end, and an electrical circuit for selectively applying an
electrical potential along each of the paths for selectively moving a plasmid first along the
first path from the bacterial well to the elution chamber, then along the second path to the
collection window at the end thereof.

L22: Entry 72 of 100 File: USPT Jun 8, 1993

DOCUMENT-IDENTIFIER: US 5217593 A
TITLE: Nucleic acid purification system and method

BSPR:
Many techniques and apparatus exist for small scale purification of plasmid DNA. The typical prior
art approach to the purification of plasmids involves a series of steps, including a collection of
cells grown in liquid culture by centrifugation, separation of the bacterial chromatic (genomic)
DNA, and cellular debris from the soluble contents of the bacteria by centrifugation of filtration,
and concentration of the plasmid DNA apart from other cellular components by alcohol or isopropanol,
absorption to solid media (i.e. ion exchange resin, glass powder, reverse phase chromatography
resin, etc.), or salt precipitation. Additional purification steps may be added to these, such as
phenol/chloroform extraction, secondary alcohol precipitation, protease or ribonuclease treatment to
further purity the plasmid DNA.

82. Document ID: US 5075430 A

L22: Entry 82 of 100 File: USPT Dec 24, 1991

US-PAT-NO: 5075430
DOCUMENT-IDENTIFIER: US 5075430 A
TITLE: Process for the purification of DNA on diatomaceous earth
DATE-ISSUED: December 24, 1991

US-CL-CURRENT: 536/25.41; 423/335, 435/803, 536/127, 536/25.42

APPL-NO: 7/ 629787
DATE FILED: December 18, 1990

PARENT-CASE:
This application is a continuation of application Ser. No. 07/288,515 filed Dec. 12, 1988, now
abandoned.

IN: Little; Michael C.

AB: This invention is directed to a process for the purification of plasmid and other
DNA, both single-stranded and double-stranded, by immobilizing the

DNA onto diatomaceous earth
in the presence of a chaotropic agent and eluting the DNA with water or low salt buffer. The
resulting purified DNA is biologically active. Also included in the invention is a process for
the immobilization of DNA onto diatomaceous earth in the presence of a chaotropic agent.

L22: Entry 82 of 100

File: USPT

Dec 24, 1991

DOCUMENT-IDENTIFIER: US 5075430 A
TITLE: Process for the purification of DNA on diatomaceous earth

BSPR:
The purification of plasmid DNA from bacterial lysates is a rate-limiting and time-consuming step in
molecular biology. The preparation of plasmid DNA for cloning and other purposes generally follows
the scheme established in Birnboim (1983, Methods in Enzymology 100:243-255) in which the cleared
bacterial lysate is applied to a cesium chloride gradient and centrifuged for 4-24 hours. This is
usually followed by the extraction and precipitation of the DNA to yield DNA that is sometimes, but
not always, free of RNA, protein and chromosomal DNA. Other methods employing cleared lysates to
prepare DNA of similar quality are ion exchange (Colpan et al., 1984, J. Chromatog. 296:339-353) and
gel-filtration (Moreau et al., 1987, Analyt. Biochem. 166:188-193) high-performance methods. While
these latter methods generally work well as alternatives for ScCl gradients, they require costly
solvent delivery systems and the reprecipitation of the isolated DNA fractions since they usually
contain salt or are too dilute, and are limited in the amount of DNA that can be prepared (<500
.mu.g) per run. Since typically 1 liter cultures of E. coli yield >2 mg of plasmid DNA plus much RNA
and protein, the capacity of the high performance methods requires multiple cycles to process these
quantities of DNA.

83. Document ID: US 5057426 A

L22: Entry 83 of 100

File: USPT

Oct 15, 1991

US-PAT-NO: 5057426
DOCUMENT-IDENTIFIER: US 5057426 A
TITLE: Method for separating long-chain nucleic acids
DATE-ISSUED: October 15, 1991

US-CL-CURRENT: 435/270; 536/25.4, 536/25.41

APPL-NO: 7/ 123698
DATE FILED: November 23, 1987

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
DE 3639949 November 22, 1986

IN: Henco; Karsten, Stichel; Arndt, Colpan; Metin

AB: A method for the separation of long-chain nucleic acids from other substances in
solutions containing nucleic acids and other materials, comprising fixing long-chain nucleic
acids in a nucleic acid-containing solution onto a porous matrix, washing the porous matrix to
separate the other substances from the long-chain nucleic acids, and removing the fixed
long-chain nucleic acids from the porous matrix is disclosed. A device for carrying out the
method of the claimed invention is also described.

L22: Entry 83 of 100

File: USPT

Oct 15, 1991

DOCUMENT-IDENTIFIER: US 5057426 A
TITLE: Method for separating long-chain nucleic acids

BSPR:
In the EP-A- 0 104 210 there has been described a method for separating nucleic acids up to plasmid
size (<10,000 base pairs= 6 million Dalton). By using the material described therein, which is
distinguished by a highly porous silica gel provided with an anion exchanger coating as employed in
HPLC chromatography that is used as a carrier, for example, prepurified plasmids may be prepared in
a highly pure state. Nevertheless, centrifugation steps and precipitation steps are necessary, which
are not suitable for application in bulk analysis and preparation, respectively. One crucial
drawback with larger molecules, for example .lambda.-phage DNA, is that during the chromatographic
separation of particles <10 .mu.m the shear forces become so high that intact molecules cannot be
recovered. This is all the more applicable to cellular DNA, which has a length many times that of
.lambda.-phage DNA.

1. Document ID: EP 376080 A, CA 2006185 A, IT 1226210 B

L26: Entry 1 of 1

File: DWPI

Jul 4, 1990

DERWENT-ACC-NO: 1990-202539
DERWENT-WEEK: 199027
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TITLE: Extn. and purification without centrifuging of DNA - from lambda phage(s) MJ13 phagemid(s),
plasmids, and cosmid(s). by exchange treatment or lysis, and macro-filtration and ultrafiltration

PRIORITY-DATA: 1988IT-0083551 (December 22, 1988)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 376080 A	July 4, 1990		000	
CA 2006185 A	June 22, 1990		000	
IT 1226210 B	December 21, 1990		000	C12K

APPLICATION-DATA:
PUB-NO
APPL-DATE
APPL-NO
DESCRIPTOR
EP 376080A
December 14, 1989
1989EP-0123171
IT 1226210B
December 22, 1988
1988IT-0083551

INT-CL (IPC): C12K 401/12; C12N 15/10; C12P 19/34

IN: SCHNEIDER, C

AB: A method for the extn. and purification of DNA, starting from lambda phages, M13
phagemids, plasmids, cosmids or other like elements is claimed comprising: (a) charging the
sample which undergoes an exchange treatment or lysis, followed by macrofiltration, (b)
treating the macrofiltrate with a precipitant and subjecting the resulting soln. to
ultrafiltration and (c) eluting and collecting the DNA fixed to the ultrafiltration support.,
For plasmids or cosmids lysis may be carried out with an alkaline soln. contg. a detergent,
e.g. sodium deoxycholate. For lambda phages and M13 phagemids the exchange treatment may be
carried out with an anionic exchange resin, e.g. DEAE and macrofiltration may be carried out
with a macroporous diaphragm supported by a nitrocellulose filter. A precipitant soln. contg. a
cationic detergent, e.g. cetyl-trimet hyl-ammonium bromide (CTAB) may be added to the
macrofiltrate to obtain a micellar complex., ADVANTAGE - The method is used to extract and
purify DNA without centrifuging steps and can be automated.

L26: Entry 1 of 1
File: DWPI
Jul 4, 1990

DERWENT-ACC-NO: 1990-202539
DERWENT-WEEK: 199027
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Extn. and purification without centrifuging of DNA - from lambda phage(s) MJ13 phagemid(s),
plasmids, and cosmid(s). by exchange treatment or lysis, and
macro-filtration and ultrafiltration

TTX:
EXTRACT PURIFICATION CENTRIFUGE DNA LAMBDA PHAGE PLASMID COSMID EXCHANGE TREAT LYSE MACRO FILTER ULTRAFILTER

3. Document ID: US 6001604 A

L28: Entry 3 of 7
File: USPT
Dec 14, 1999

US-PAT-NO: 6001604
DOCUMENT-IDENTIFIER: US 6001604 A
TITLE: Refolding of proinsulins without addition of reducing agents
DATE-ISSUED: December 14, 1999

US-CL-CURRENT: 435/69.4; 435/68.1, 435/69.7, 530/344, 530/412, 530/414

APPL-NO: 8/ 967867
DATE FILED: November 12, 1997

PARENT-CASE:
This application is a continuation of U.S. Ser. No. 08/367,454, filed Dec. 29, 1994, now abandoned,
which is a continuation-in-part of U.S. Ser. No. 08/175,298, filed Dec. 29, 1993 now abandoned.

IN: Hartman; Jacob R., Mendelovitz; Simona, Gorecki; Marian

AB: An improved and efficient process for the production of recombinant human insulin by
folding of a proinsulin hybrid polypeptide is provided.

L28: Entry 3 of 7
File: USPT
Dec 14, 1999

DOCUMENT-IDENTIFIER: US 6001604 A
TITLE: Refolding of proinsulins without addition of reducing agents

DEPR:
Alternatively, the SOD-proinsulin hybrid polypeptide expressed by plasmid pBAST-R was purified to
near homogeneity by dissolution in 8M urea, 20 mM Dithiothreitol, 50 mM NaAcetate, pH 5, and by
ultrafiltration through a series of 100 kD and 50 kD membranes (Filtron). The hybrid polypeptide was
concentrated on a 10 kD membrane and precipitated with (NH.sub.4).sub.2 SO.sub.4 at 40% saturation.

6. Document ID: EP 517515 A2, EP 517515 A3, JP 04360686 A

L28: Entry 6 of 7
File: DWPI
Dec 9, 1992

DERWENT-ACC-NO: 1992-408948
DERWENT-WEEK: 199250
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TITLE: Purifying plasmid DNA and/or cosmid DNA from microorganism cells - by lysing cells, filtering
to remove insoluble material and ultrafiltrat ion to condense the DNA, avoiding toxic reagent usage

PRIORITY-DATA: 1991JP-0159436 (June 4, 1991)

PATENT-FAMILY:
PUB-NO
PUB-DATE
LANGUAGE
PAGES
MAIN-IPC
EP 517515 A2
December 9, 1992
E
005
C12N015/10
EP 517515 A3
June 16, 1993
000
C12N015/10
JP 04360686 A
December 14, 1992
004
C12N015/10

APPLICATION-DATA:
PUB-NO
APPL-DATE
APPL-NO
DESCRIPTOR
EP 517515A2
June 4, 1992
1992EP-0305119
EP 517515A3
June 4, 1992
1992EP-0305119
JP04360686A
June 4, 1991
1991JP-0159436

INT-CL (IPC): B01D 61/00; C12N 1/06; C12N 15/10

IN: NISHI, A, OGAWA, K

AB: Purifying plasmid DNA and/or cosmid DNA comprises (a) lysing microorganism cells
contg. the plasmid DNA and/or cosmid DNA; (b) filtering the resultant lysate through a membrane
filter to remove any insoluble material; and (c) subjecting the filtrate to ultrafiltration to
thereby condense the DNA., The method may further comprise removing RNA originating from the
cells by passing a soln. contg. an RNase and/or an aq. alkaline metal hydroxide through the
ultrafilter. The cells may be lysed by treatment with an aq. alkaline hydroxide soln., such as
NaOH and/or KOH, and/or a surfactant, such as sodium dodecyl sulphate (SDS)., USE/ADVANTAGE -
Using the method, plasmid DNA and/or cosmid DNA can be purified to high purity from
microorganism cells by simple operations without using toxic reagent

L28: Entry 6 of 7
File: DWPI
Dec 9, 1992

DERWENT-ACC-NO: 1992-408948
DERWENT-WEEK: 199250

TITLE: Purifying plasmid DNA and/or cosmid DNA from microorganism cells - by lysing cells, filtering
to remove insoluble material and ultrafiltrat ion to condense the DNA, avoiding toxic reagent usage

TT X:
PURIFICATION PLASMID DNA COSMID DNA MICROORGANISM CELL LYSE CELL FILTER REMOVE INSOLUBLE MATERIAL
ULTRAFILTER CONDENSATION DNA AVOID TOXIC REAGENT